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Pharmacogenetic Interactions Between Angiotensin-Converting Enzyme Inhibitor Therapy and the Angiotensin-Converting Enzyme Deletion Polymorphism in Patients With Congestive Heart Failure

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OBJECTIVES We evaluated the interaction of angiotensin-converting enzyme (ACE) inhibitor therapy with the effect of the ACE D/I polymorphism on heart failure survival.

BACKGROUND The ACE deletion allele, ACE-D, is associated with increased ACE activity. The utilization of ACE genotyping to predict the impact of ACE inhibitor dose has not been previously evaluated.

METHODS We prospectively studied 479 subjects with systolic dysfunction (left ventricular ejection fraction 0.25 ± 0.08). Subjects were divided on the basis of ACE inhibitor therapy into low dose (≤50% of target dose, n = 227), standard (high) dose (>50%, n = 201), or those receiving angiotensin receptor antagonists (n = 51). Patients were genotyped for the ACE D/I polymorphism, followed to the end point of death or cardiac transplantation, and transplant-free survival compared by genotype.

RESULTS The ACE-D allele was associated with an increased risk of events (p = 0.026). In analysis by ACE inhibitor dose, this effect was primarily in the low-dose group (1-year percent event-free survival: II/ID/DD = 86/77/71, 2-year = 79/66/59, p = 0.032). In the standard-dose group, the impact was markedly diminished (1-year: II/ID/DD = 91/81/80, 2-year: 77/70/71, p = 0.64). The impact of beta-blockers and high dose ACE inhibitors was greatest in subjects with the ACE DD genotype (p = 0.001) and was less apparent with the II and ID genotypes (p = 0.38).

CONCLUSIONS Higher doses of ACE inhibitors diminished the impact of the ACE-D allele, and the benefits of beta-blockers and high-dose ACE inhibitors appeared maximal for DD patients. Determination of ACE genotype may help target therapy for patients with heart failure. (J Am Coll Cardiol 2004;44:2019–26) © 2004 by the American College of Cardiology Foundation

Treatment with angiotensin-converting enzyme (ACE) inhibitors remains the cornerstone of heart failure therapy (1). More recently, beta-adrenergic receptor antagonists (beta-blockers) have been demonstrated to improve survival in chronic heart failure (2,3). The efficacy of these therapies has been demonstrated in multicenter trials for large populations; however, significant heterogeneity may exist in the benefits to individual subjects. Pre-treatment assessment of therapeutic efficacy would allow tailoring of therapy to optimize outcomes, but at present the tools to define which individuals obtain maximal benefit are limited.

Despite the benefits of ACE inhibition, efforts to further improve heart failure survival by increasing neurohormonal blockade, either with high-dose ACE inhibitors (4) or the addition of angiotensin receptor blockers (ARB) (5), have met with limited success. The role of genetic heterogeneity in modulating the effectiveness of these pharmacologic interventions has not been explored. A common polymorphism exists in intron 16 of the ACE gene in which the two alleles differ on the presence or absence of a 287 base-paired insertion (I = insertion; D = deletion) (6,7). The D allele is associated with higher ACE activity (8–10) and has been previously associated with poorer survival for patients with congestive heart failure (11). The ACE DD genotype delineates a large patient subset, one-third of the general population, known to have higher levels of angiotensin activation. We have previously reported a pharmacogenetic interaction of the ACE D/I polymorphism with beta-blocker therapy (12); however, the impact of ACE inhibitor dose has not been evaluated. In a population with heart failure, we sought to evaluate the pharmacogenetic interaction of the ACE D/I polymorphism with the impact of ACE inhibitor dose on clinical outcomes, and to explore the potential use of ACE genotype to target therapy.

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Abbreviations and Acronyms

ACE = angiotensin-converting enzyme
ARB = angiotensin receptor blocker
CI = confidence interval
LVEF = left ventricular ejection fraction
NYHA = New York Heart Association

METHODS

Study population. The Institutional Review Board of the University of Pittsburgh Medical Center approved this study. A series of 479 patients with heart failure resulting from systolic dysfunction referred to the Cardiomyopathy Clinic at the University of Pittsburgh Medical Center were recruited into a study of Genetic Risk Assessment of Cardiac Events (GRACE) between April 1996 and January 2001. Preliminary analysis of the first 328 subjects has been previously reported (12). Informed consent was obtained and peripheral blood drawn for deoxyribonucleic acid (DNA) isolation and genotyping. At entry, demographic information, New York Heart Association (NYHA) functional class, and medical therapy were recorded. Patients were prospectively followed to an end point of either death or cardiac transplantation. Medical personnel participating in management and follow-up were unaware of the genotype status of individual subjects.

In all patients, the most recent clinical assessment demonstrated left ventricular systolic dysfunction, defined as left ventricular ejection fraction (LVEF) $\leq$0.45 (n = 449) or qualitative assessment documenting moderate to severe left ventricular dysfunction (n = 30). The LVEF was estimated by radionuclide scan in 177 patients (37.0%), left ventricular angiography in 27 (5.6%), and by echocardiography in 275 (57.4%). Patients with angiographic evidence of coronary disease (defined as >50% stenosis of a major epicardial coronary artery), or a noninvasive assessment consistent with ischemia or previous infarction were classified as ischemic.

Genotyping of the ACE polymorphism. Genomic DNA was extracted from peripheral blood with a Pure Gene Kit, Gentra Systems, Inc. (Minneapolis, Minnesota). The ACE genotyping was performed using the method of Lindpaintner et al. (13); primers 5′GCC CTG CAG GTG TCT GCA GCA TGT 3′ and 5′ GGA TGG CTC TCC CCG CCT TGT CTC 3′ were used to amplify the D and I alleles, resulting in 319 base pair (bp) and 597-bp products, respectively. Polymerase chain reactions were run for 35 cycles: 30 s at 94°, 45 s at 56°, and 2 min at 72°. The product was subjected to electrophoresis in a 1.5% agarose gel and stained with ethidium bromide. Given preferential amplification of the D allele in heterozygous samples, samples found to have the DD genotype were re-amplified using insertion-specific primers: 5′ TGG GAC CAC AGC GCC CGC CAC TAC 3′, and 5′ TCG CCA GCC CTC CCA TGC CCA TAA 3′ and identical polymerase chain reaction conditions except for an annealing temperature of 67°. Evaluation of these products on a 1.5% agarose gel revealed a 335-bp product in the presence of an I allele.

Medical therapy classification. For both the beta-blocker and ACE dose, patients were categorized on the basis of their therapy at time of study entry. For analysis of the effect of ACE inhibition, subjects whose daily dose of ACE inhibitor therapy at the time of study entry was >50% of target dose based on published guidelines (14–16) were classified as "high-dose," whereas those whose daily dose was $\leq$50% were classified as "low-dose." Patients on angiotensin receptor antagonists were excluded from the ACE dose analysis but included in the overall outcome analysis.

Follow-up and outcomes analysis. Patients were followed prospectively to the end point of death or cardiac transplantation. The effect of the ACE-D allele on events-free survival was analyzed for the entire cohort, and then separately in subsets based on medical therapy class (beta-blockers vs. no beta-blockers; ACE low dose vs. high dose). For the analysis of the impact of therapy within genotype class, the four neurohormonal treatment strategies (1: beta-blockers plus high-dose ACE inhibitor; 2: beta-blockers plus low dose; 3: no beta-blockers plus high dose; and 4: no beta-blockers plus high dose) were analyzed for the subjects with the ACE DD genotype, and then for the remainder (II and ID subjects combined).

Statistical analysis. Results are presented as mean values ± SD. Continuous baseline characteristics were compared nonparametrically based on ordered genotype status using the Jonckheere-Terpstra test (17), whereas these comparisons were made between two groups using the Wilcoxon rank-sum test. The Pearson chi-square test was used to compare distributions of binary variables between two groups. The Mantel-Haenszel chi-square test was used to compare distributions of all categorical variables by ordered genotype status, as well as of ordered categorical variables (NYHA functional class) between two groups. For outcome analysis, Kaplan-Meier event-free survival curves were constructed. The log-rank test was used for comparison of survival curves. In the case of multiple ordered subgroups (such as those defined by genotype status), a log-rank test incorporating linear trend across genotype levels was used for testing equality of event-free survival. Cox regression analysis was used to quantify the relative risk of events over time; the significance of reported coefficients was assessed by the Wald test. For each analysis, the statistical interaction of drug therapy with ACE genotype class was assessed using a Cox regression model with linear ordering of relevant categories. Specifically, for analysis of survival by ACE genotype, gene dose was utilized as the ordered variable (assuming an intermediate effect for heterozygotes), whereas for analysis of the impact of therapy within genotype class, degree of neurohormonal blockade was used.

Table 1. Patient Characteristics by Genotype

	II (n = 89)	ID (n = 243)	DD (n = 147)	All Patients (n = 479)
Age (yrs)	55.4 ± 12.2	55.4 ± 12.1	56.5 ± 11.7	55.7 ± 12.0
Female (%)	25.8	30.9	25.9	28.4
Caucasian (%)	91.0	91.4	90.5	91.0
NYHA class, I/II/III/IV (%)	0.0/44.9/48.3/6.7	4.5/44.9/48.1/2.5	1.4/40.1/50.3/8.2	2.7/43.4/48.9/5.0
Ischemic (%)	48.3	46.1	55.1	49.3
LVEF (n = 449)	0.25 ± 0.07	0.24 ± 0.09	0.25 ± 0.09	0.25 ± 0.08
Na (mg/dl) (n = 316)	138.2 ± 3.4	138.9 ± 3.5	138.8 ± 3.7	138.7 ± 3.6
Cr (mg/dl) (n = 318)	1.32 ± 0.50	1.34 ± 0.57	1.40 ± 0.77	1.36 ± 0.64
Initial medical therapy				
ACE inhibitor (%)	84.3	86.0	81.0	84.1
ARB (%)	9.0	9.9	12.9	10.6
Beta-blocker (%)	41.6	42.0	42.9	42.2

Values are mean ± SD or percent of patients. No significant differences were detected between the three genotype subgroups.
ACE = angiotensin-converting enzyme; ARB = angiotensin receptor blocker; Cr = serum creatinine; LVEF = left ventricular ejection fraction; Na = serum sodium; NYHA = New York Heart Association.

RESULTS

Baseline demographics and clinical characteristics. The demographic and baseline clinical characteristics by genotype are listed in Table 1. The mean age of the cohort was 55.7 ± 12.0 years. The population was 72% male, 91% white, and 49% ischemic. Genotyping classified 18.6% of patients (n = 89) as homozygotes for the I allele, 50.7% (n = 243) as heterozygotes, and 30.7% (n = 147) as homozygotes with D allele. No significant differences in medical therapy were detected among the three genotype subgroups.

Classification by medical therapy. HIGH- VERSUS LOW-DOSE ACE INHIBITOR THERAPY. Based on ACE inhibitor dose at entry, 42% (n = 201) of subjects were classified in the high-dose subset. This treatment group could be more accurately characterized as the "standard" dose therapy subset as the majority were at (n = 121, 60%) or above (n = 56, 28%) target doses defined by heart failure treatment guidelines. The remaining subjects in the higher dose treatment subset were on 75% (n = 17, 9%) or 67% (n = 7, 3%) of the target dose. Forty-seven percent of subjects (n = 227) were classified in the low-dose group, including 5% (n = 25) on neither an ACE inhibitor nor an ARB. The remaining subjects (n = 51, 11%) were on ARBs. Nine percent of subjects (n = 43) were treated with the aldosterone receptor antagonist (spironolactone) at the time of entry.

The demographics and clinical characteristics by dose class are listed in Table 2. Patients on low-dose therapy were slightly older (mean age 56.5 ± 12.1 years vs. 54.1 ± 12.2 years, p = 0.050). In addition, the percentage of patients with an ischemic etiology was higher in the low-dose group than in the high-dose group (56.8% vs. 39.3%, p < 0.001). The NYHA functional class, LVEF, and the percentage of patients receiving beta-blockers were similar between groups (Table 2). In addition, the percentage of patients with systolic or diastolic hypertension and the mean diastolic and systolic blood pressures were all comparable in the higher dose and low-dose groups. The majority of subjects were taking either captopril, enalapril, or lisinopril; mean doses for the low- and high-dose class are listed in Table 3.

For 85% (299 of 359) of those subjects alive and not transplanted at one year, medical therapy was reassessed. At one year, 13% of subjects had moved from the low to the higher dose class, 8% from higher to low dose, whereas 5% switched from an ACE inhibitor to an ARB. The majority of subjects (74%) remained in the same dosage class at one year, suggesting that in early follow-up entry dosage was reflective of treatment strategy.

BETA-BLOCKERS. Overall 202 patients (42%) were receiving beta-blocker therapy at study entry and 277 (58%) were not taking beta-blockers. Of those receiving therapy, 55% were taking carvedilol (mean daily dose 31 ± 24 mg), 39% metoprolol (59 ± 34 mg), and 6% other. Patients taking beta-blockers were slightly younger (mean age 54.4 ± 12.2 vs. 56.7 ± 11.7 years, p = 0.03) and had a higher mean LVEF (0.26 ± 0.09 vs. 0.24 ± 0.08, p = 0.03).

Outcomes by ACE genotype. EVENT-FREE SURVIVAL: ENTIRE COHORT. For patients alive and transplant-free at last follow-up, median follow-up was 33 months (range 3 to 62 months). During the course of follow-up there were 194

Table 2. Patient Characteristics by ACE Inhibitor Dose

	High Dose (n = 201)	Low Dose (n = 227)
Age (yrs)	54.1 ± 12.2	56.5 ± 12.1*
Female (%)	27.9	29.5
Caucasian (%)	90.0	92.1
NYHA class, I/II/III/IV (%)	4.5/44.3/46.8/4.5	1.3/44.5/48.5/5.7
Ischemic (%)	39.3	56.8†
Beta-blocker (%)	41.8	42.7
LVEF (n = 402)	0.25 ± 0.08	0.24 ± 0.08
Na (mg/dl) (n = 275)	138.7 ± 3.5	138.6 ± 3.6
Cr (mg/dl) (n = 277)	1.35 ± 0.57	1.38 ± 0.71
BP sys (mm Hg) (n = 427)	114 ± 17	112 ± 17
BP dia (mm Hg) (n = 426)	71 ± 11	71 ± 10

Values are mean ± SD or percent of patients. *p = 0.050; †p < 0.001 for significance of the distributions of this variable between the two groups.
BP dia = diastolic blood pressure; BP sys = systolic blood pressure; other abbreviations as in Table 1.

Table 3. ACE Inhibitor Utilization

Drug (Target*)	Overall Cohort n (%)	Overall Cohort Mean Dose (mg)	Low-Dose Group n (%)	Low-Dose Group Mean Dose (mg)	High-Dose Group n (%)	High-Dose Group Mean Dose (mg)
Enalapril (20 mg)	146 (36.2)	17 ± 13	63 (31.2)	8 ± 3	83 (41.3)	24 ± 13
Captopril (150 mg)	122 (30.3)	82 ± 49	79 (39.1)	51 ± 23	43 (21.4)	129 ± 26
Lisinopril (20 mg)	101 (25.1)	18 ± 12	49 (24.3)	8 ± 3	52 (25.9)	27 ± 11
Others	34 (8.4)		11 (5.4)		23 (11.4)	

*References 14, 15, and 16; mean dose indicates total daily dose.
ACE = angiotensin-converting enzyme.

events, including 137 deaths and 57 transplants. The majority of the deaths were cardiac in etiology (94%), with heart failure deaths accounting for 46% and sudden death 39%. The cause of death did not differ significantly by genotype subset. The D allele was associated with poorer transplant-free survival (1-year percent transplant-free by genotype II/ID/DD = 89/80/74: 2-year = 77/69/62; p = 0.026 (Fig. 1A). Corresponding Cox regression analysis with the II genotype as the reference category found a relative risk for heterozygotes of 1.28 (95% confidence interval [CI] 0.86 to 1.92, p = 0.23) and a significantly increased risk among patients with the DD genotype of 1.58 (95% CI 1.04 to 2.40, p = 0.032). When analyzed separately by etiology of heart failure, the effect of the ACE-D allele on survival appeared similar in non-ischemic and ischemic subsets (non-ischemic: 1-year percent transplant-free by genotype II/ID/DD = 93/84/81, 2-year = 87/74/71, p = 0.35; ischemic: 1-year = 84/76/69, 2-year = 67/63/56, p = 0.08).

BETA-BLOCKER THERAPY. Analysis demonstrated a significant pharmacogenetic interaction with beta-blocker utilization, as previously reported in a smaller subset (12). The adverse effect of the ACE-D allele on event-free survival was strongly evident in subjects not receiving beta-blockers (1-year percent transplant-free survival II/ID/DD = 88/78/63; 2-year = 80/65/51; p = 0.004) (Fig. 1B) with a relative risk among heterozygotes of 1.28 (95% CI 0.78 to 2.10, p = 0.33) and among the DD homozygotes of 1.98 (95% CI 1.18 to 3.30, p = 0.009). For patients taking beta-blockers, the ACE deletion appeared to have no impact on outcomes (1-year II/ID/DD = 89/84/89; 2-year = 73/77/78; p = 0.97) (Fig. 1C).

ACE INHIBITOR HIGH VERSUS LOW DOSE. The interaction of ACE dose and genetic risk was evaluated through a comparison of the impact of the ACE-D allele on outcomes in the two ACE dose subsets. The adverse effect of the D allele was primarily evident in the low-dose therapy group (1-year percent transplant-free survival II/ID/DD = 86/77/71; 2-year = 79/66/59; p = 0.032) with a borderline increase in the relative risk for heterozygotes to 1.67 (95% CI 0.86 to 3.21; p = 0.13) and a significant increase among patients with the DD genotype to 2.07 (95% CI 1.06 to 4.05, p = 0.03) (Fig. 2A). The adverse impact of the D allele on outcomes was markedly diminished for those in the high-dose group (1-year II/ID/DD = 91/81/80; 2-year = 77/70/71; p = 0.64) (Fig. 2B).

Although the power was limited for analysis by etiology, the impact of ACE inhibitor dose on the effect of the ACE-D allele on survival appeared similar in ischemic and non-ischemic subsets (low-dose ACE inhibitors, non-ischemic: 1-year percent transplant-free by genotype II/ID/DD = 93/81/79, 2-year = 93/73/65, p = 0.08; low-

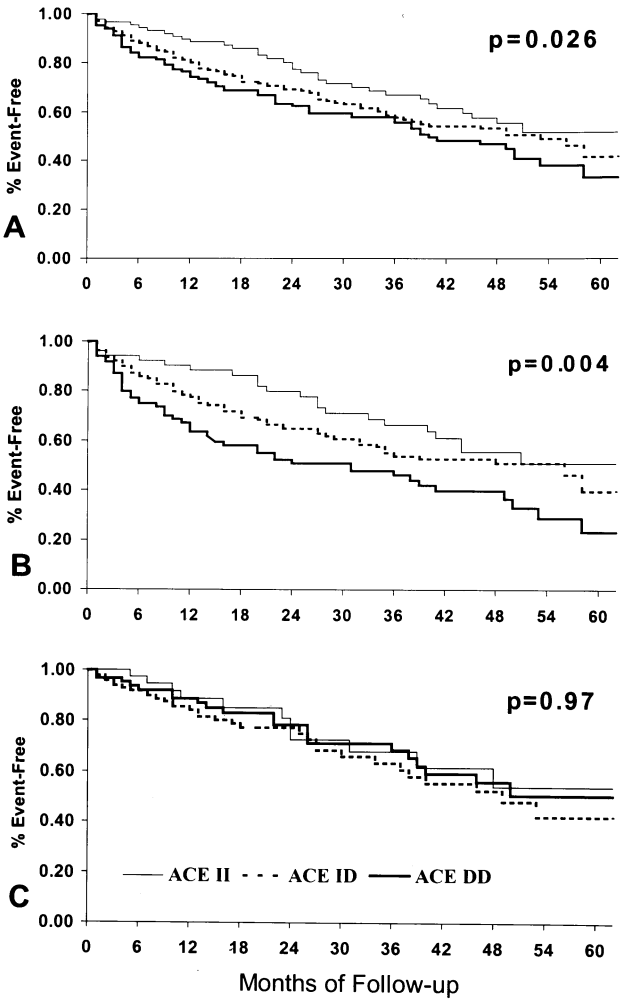

Figure 1. (A) Overall transplant-free survival by angiotensin-converting enzyme (ACE) genotype (n = 479, p = 0.026). **(B)** Transplant-free survival by ACE genotype with no beta-blocker therapy (n = 277, p = 0.004). **(C)** Transplant-free survival by ACE genotype with beta-blocker therapy (n = 202, p = 0.97).

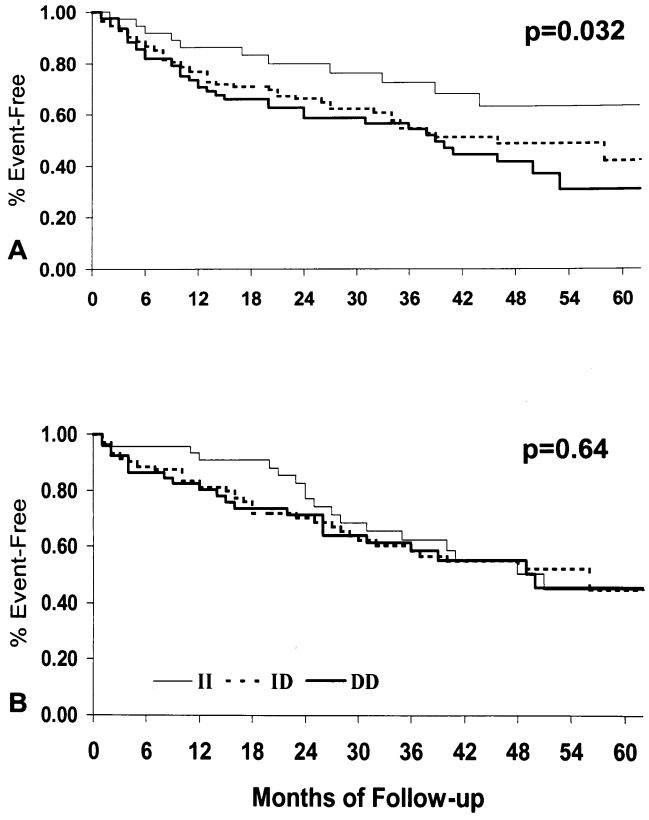

Figure 2. (A) Transplant-free survival by angiotensin-converting enzyme (ACE) genotype: low-dose ACE inhibitor (n = 227, p = 0.032). **(B)** Transplant-free survival by ACE genotype: high-dose ACE inhibitor (n = 201, p = 0.64).

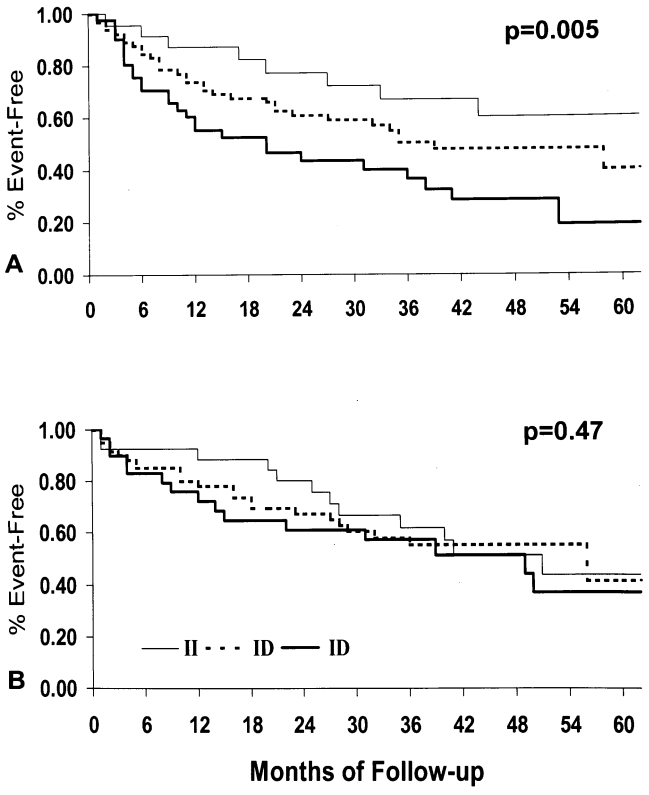

Figure 3. (A) Transplant-free survival by angiotensin-converting enzyme (ACE) genotype: low-dose ACE inhibitor therapy with no beta-blockers (n = 130, p = 0.005). **(B)** Transplant-free survival by ACE genotype: high-dose ACE inhibitor therapy with no beta-blockers (n = 117, p = 0.47).

dose ischemic: 1-year = 81/73/70, 2-year = 71/60/54, p = 0.22; high-dose ACE inhibitors, non-ischemic: 1-year percent transplant-free by genotype II/ID/DD = 96/84/86, 2-year = 87/73/81, p = 0.81; higher dose ischemic: 1-year = 82/76/73, 2-year = 60/65/58, p = 0.44).

EFFECT OF ACE DOSE IN SUBJECTS NOT RECEIVING BETA-BLOCKERS. The analysis of ACE dose was repeated in the subset of patients not taking beta-blockers (excluding 30 patients taking ARBs, n = 247). In the subgroup receiving minimal neurohormonal blockade (low-dose ACE and no beta-blockers, n = 130), the effects of the D allele on outcomes were dramatically increased (1-year II/ID/DD = 87/74/55; 2-year = 77/61/43, p = 0.005) (Fig. 3A), with a relative risk for heterozygotes of 1.62 (95% CI 0.74 to 3.52, p = 0.23) and for DD homozygotes of 2.75 (95% I 1.25 to 6.08, p = 0.012). High-dose ACE inhibitor therapy (n = 117) still diminished the impact of the ACE-D allele (1-year = 88/78/72; 2-year = 80/67/61, p = 0.47) (Fig. 3B).

Outcomes by treatment strategy. RELATIVE RISK OF EVENT BY THERAPY. Analysis of the entire cohort suggests poorer event-free survival for subjects with ACE DD genotype; however, this subset also appears to obtain maximal benefit from medical intervention. The relative risks of events (death or transplant) by therapy are listed in Table 4.

Overall, beta-blocker therapy was associated with a significant reduction in event rate (29%, p = 0.03). Within genotype class, beta-blockers dramatically reduced the event rate for DD subjects (53%, p = 0.004), but not for patients who were ID (15%, p = 0.46) or II (3%, p = 0.94). Overall, only a modest nonsignificant reduction in event-free survival was evident with high-dose ACE therapy when compared with low-dose (14%, p = 0.33). However, a similar trend toward greater benefit is evident in the DD subgroup, particularly in those not treated with beta-blockers (38%, p = 0.14).

IMPACT OF HEART FAILURE THERAPY ON TRANSPLANT-FREE SURVIVAL. Evaluation of treatment strategy within the DD genotype class suggests that increasing neurohormonal blockade is of significant clinical benefit in this subset. Event-free survival was poorest on low-dose ACE inhibitors alone (1-year and 2-year percent transplant-free survival = 55/43), intermediate on high-dose ACE inhibitors alone (1-year/2-year = 72/61), and best for those receiving beta-blockade regardless of ACE inhibitor dose (beta-blockers plus low-dose ACE = 89/77; beta-blockers plus high-dose ACE = 91/86). An ordered comparison of freedom from event curves (Fig. 4A) of increasing neurohormonal blockade (1 = low-dose ACE alone; 2 = high-dose ACE alone; 3 = low-dose plus beta-blockers; and 4 =

Table 4. Relative Risk of Events by Treatment

	Odds Ratio	95% Confidence Interval	p Value
Beta-blockers			
Overall	0.71	(0.53, 0.96)	0.027
ACE genotype			
II	0.97	(0.47, 2.03)	0.94
ID	0.85	(0.55, 1.31)	0.46
DD	0.47	(0.28, 0.79)	0.004
High-dose ACE inhibitors			
Overall	0.86	(0.64, 1.16)	0.33
ACE genotype			
II	1.35	(0.63, 2.85)	0.44
ID	0.88	(0.57, 1.35)	0.55
DD	0.75	(0.44, 1.27)	0.29
High-dose ACE inhibitors (subject not on beta-blockers)			
Overall	0.88	(0.56, 1.15)	0.24
ACE genotype			
II	1.31	(0.54, 3.22)	0.55
ID	0.86	(0.51, 1.47)	0.59
DD	0.62	(0.33, 1.17)	0.10

ACE = angiotensin-converting enzyme.

high-dose plus beta-blockers) demonstrates a significant benefit of increasing therapy for subjects with the DD genotype ($p < 0.001$).

In contrast, for the remaining subjects (ID and II combined), neurohormonal treatment strategy appeared to have less impact on event-free survival (low-dose ACE alone 1-year and 2-year percent transplant-free survival = 77/65, high dose alone = 81/71, low-dose plus beta-blockers = 82/78, high-dose plus beta-blockers = 88/72; p = 0.38) (Fig. 4B). The statistical interaction of therapy with ACE genotype was of greater magnitude when ACE inhibitor dose and beta-blocker use were considered jointly (overall interaction significant, p = 0.03) (Fig. 4) than for analysis of either beta-blocker use (p = 0.08) (Figs. 1B and 1C) or ACE dose alone (p = 0.25, Fig. 2; p = 0.16, Fig. 3).

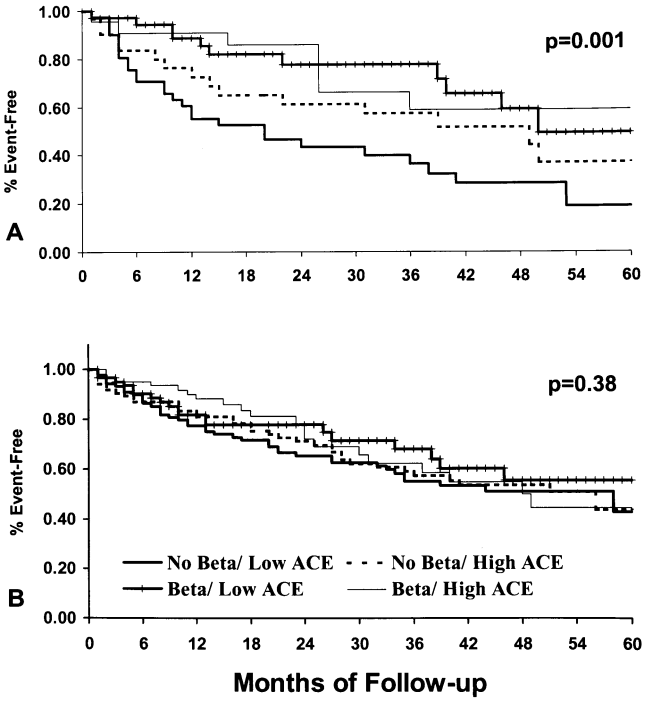

Figure 4. **(A)** Transplant-free survival by treatment strategy, DD genotype only (n = 128, p = 0.001). **(B)** Transplant-free survival by treatment strategy, ID and II genotypes combined (n = 300, p = 0.38). ACE = angiotensin-converting enzyme; Beta = beta-blockers.

DISCUSSION

This study demonstrates a pharmacogenetic interaction between the ACE D/I polymorphism and the impact of ACE inhibitor dose on heart failure survival. In addition, this study reconfirms in a larger cohort the previously reported interaction between the ACE-D allele and the effectiveness of beta-blocker therapy. Of the estimated 5 million Americans with heart failure (18), approximately 1.5 to 2 million are predicted to have the ACE DD genotype. These findings suggest that this genetic subset obtains the maximal survival benefit from increasing levels of neurohormonal inhibition, and that this common polymorphism can be potentially utilized to tailor heart failure therapy.

High-dose ACE inhibitor therapy has been shown to reduce hospitalization (19) and improve functional capacity (20) for patients with heart failure; however, efforts to further augment improvements in survival with high-dose therapy have not been successful. The Assessment of Treatment with Lisinopril And Survival (ATLAS) trial (4) compared the effects of low-dose (2.5 to 5 mg daily of lisinopril) versus high-dose ACE inhibitor therapy (32.5 to 35 mg daily) in a prospective randomized study of more than 3,000 subjects with heart failure. Although a significant reduction in heart failure hospitalization was evident with high-dose therapy, the impact on survival was modest (8% reduction in the risk of death, p = 0.128) and comparable to the current study despite a much greater dose differential in the ATLAS trial. In contrast to the higher

doses used in the randomized trial, the impact of dose may be underestimated in the current study as the majority in the "high" subset were actually on standard-dose therapy. However, the differential treatment effects by genotype class in our study suggest that the genetic heterogeneity of the ACE gene locus may have contributed to the absence of survival benefit in the randomized trial.

Given the absence of randomization to the medical therapy subsets, these results must be viewed as exploratory in nature. The low-dose and standard ('high') dose groups appear comparable (Table 2), although ischemic etiology was more prevalent in the low-dose group. Subset analysis by etiology suggests that the increased prevalence of coronary artery disease did not influence the greater impact of the D allele in the low-dose group. Neurohormonal markers of ACE inhibition were not assessed during this investigation, and therefore the study can not objectively compare the degree of ACE inhibition between the high-dose and low-dose groups. However, despite greater reductions in ACE activity, high-dose ACE inhibitors do not have a greater effect on neurohormone levels (21,22). Recently, a newly discovered homologue of the ACE gene has been identified which has significant cardiac expression (23) and appears to inactivate angiotensin II. The role of this second ACE gene in the regulation of cardiac function and the implication for future genetic targeting strategies remain unknown.

Combination therapy with an ARB may be the preferred method of blockade, both in terms of ventricular remodeling (24) and reductions in sympathetic activity (25). The Valsartan Heart Failure Trial (VAL-HeFT) (5) evaluated the addition of the ARB valsartan to the standard regimen and found improvements in a combined end point (death or re-hospitalization) but no significant benefit for survival alone. Subset analysis suggested that the impact of valsartan on survival differed markedly depending on concordant therapy, with decreased survival in the subset on an ACE inhibitor, ARB, and beta-blocker. This has led to speculation that excessive neurohormonal blockade may actually be harmful (26) and has emphasized the need for improved methodologies for targeting therapies.

The competitive interaction between beta-blockers and ACE inhibitors evident in clinical trials was also demonstrated in this analysis. The interaction of ACE inhibitor dose and ACE genetic background was primarily in subjects not receiving beta-blockers (Fig. 3), consistent with previous reports (12,27) that beta-blockers diminish the impact of the ACE-D allele on heart failure survival. The pharmacogenetic interaction of the ACE-D allele and the impact of therapy was statistically more powerful when both beta-blocker usage and ACE inhibitor dose were included in the analysis (Fig. 4). While an analysis of beta-blocker dose would be of interest, the Carvedilol Or Metoprolol European Trial (COMET) has demonstrated potential differences in the clinical impact of carvedilol and metoprolol tartrate (28). This suggests beta-blockers may not be interchangeable and makes the pooling of beta-blocker data more complex. This study is therefore limited in its ability to address the impact of beta-blocker dose.

The current data do support the pharmacogenetic hypothesis that the ACE D/I polymorphism modulates the effect of heart failure therapy in a manner consistent with the known effect of the D allele on ACE activity. This hypothesis can only be truly evaluated in the context of a clinical trial, and these findings should be confirmed in randomized studies before the development of pharmacogenetic targeting strategies. The results of this study strongly support the need for randomized pharmacogenetic trials and suggest that genetic assessment of therapeutic efficacy should be incorporated into all future pharmaceutical trials. If the current findings are confirmed, one-third of heart failure patients with the DD genotype could be selected for more aggressive neurohormonal therapy. Further investigations will hopefully clarify the role of ACE genotyping in the clinical management of patients with heart failure.

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REFERENCES

1. Braunwald E. ACE inhibitors—a cornerstone of the treatment of heart failure. N Engl J Med 1991;325:351–3.
2. Packer M, Bristow MR, Cohn JN, et al. The effect of carvedilol on morbidity and mortality in patients with chronic heart failure. N Engl J Med 1996;334:1349–55.
3. The MERIT-HF Study Group. Effect of metoprolol CR/XL in chronic heart failure: Metoprolol CR/XL Randomized Intervention Trial in congestive Heart Failure (MERIT-HF). Lancet 1999;353: 2001–7.
4. Packer M, Poole-Wilson PA, Armstrong PW, et al. Comparative effects of low and high doses of the angiotensin-converting enzyme inhibitor, lisinopril, on morbidity and mortality in chronic heart failure. Circulation 1999;100:2312–8.
5. Cohn JN, Gognoni G, for the Valsartan Heart Failure Trial Investigators. A randomized trial of the angiotensin-receptor blocker valsartan in chronic heart failure. N Engl J Med 2001;345:1667–75.
6. Rigat B, Hubert C, Alhenc-Gelas F, Cambien F, Corvol P, Soubrier F. An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. J Clin Invest 1990;86:1343–6.
7. Rigat B, Hubert C, Corvol P, Soubrier F. PCR detection of the insertion/deletion polymorphism of the human angiotensin converting enzyme gene (DCP) (dipeptidyl carboxypeptidase 1). Nucleic Acids Res 1992;20:1433.
8. Tiret L, Rigat B, Visvikis S, et al. Evidence, from combined segregation and linkage analysis, that a variant of the angiotensin I-converting enzyme (ACE) gene controls plasma ACE levels. Am J Hum Genet 1992;51:197–205.
9. Danser AH, Derkx FH, Hense HW, Jeunemaitre X, Riegger GA, Schunkert H. Angiotensinogen (M235T) and angiotensin-converting enzyme (I/D) polymorphisms in association with plasma renin and prorenin levels. J Hypertens 1998;16:1879–83.
10. Ihnken R, Verho K, Gross M, Marz W. Deletion polymorphism of

the angiotensin I-converting enzyme gene is associated with increased plasma angiotensin-converting enzyme activity but not with increased risk for myocardial infarction and coronary artery disease. Ann Intern Med 1996;125:19–25.
11. Andersson B, Sylven C. The DD genotype of the angiotensin-converting enzyme gene is associated with increased mortality in idiopathic heart failure. J Am Coll Cardiol 1996;28:162–7.
12. McNamara DM, Holubkov R, Janosko K, et al. Pharmacogenetic interactions between β-blocker therapy and the angiotensin-converting enzyme deletion polymorphism in patients with congestive heart failure. Circulation 2001;103:1644–8.
13. Lindpaintner K, Pfeffer MA, Kreutz R, et al. A prospective evaluation of an angiotensin-converting-enzyme gene polymorphism and the risk of ischemic heart disease. N Engl J Med 1995;332:706–11.
14. Roe CM, Motheral BR, Teitelbaum F, et al. Angiotensin-converting enzyme inhibitor compliance and dosing among patients with heart failure. Am Heart J 1999;138:818–25.
15. Konstam MA, Dracup K, Baker DW, et al. Heart failure: evaluation and care of patients with left ventricular dysfunction. Clinical practice guideline no. 11. Rockville, MD: Agency for Health Care Policy and Research, Public Health Service, U.S. Department of Health and Human Services, 1994:AHCPR publication No. 94-0612.
16. Chin MH, Wang JC, Zhang JX, et al. Utilization and dosing of angiotensin-converting enzyme inhibitors for heart failure. J Gen Intern Med 1997;12:563–6.
17. Jonckheere AR. A distribution-free k-sample test against ordered alternatives. Biometrika 1954;41:133–45.
18. Hunt SA, Baker DW, Chin MH, et al. ACC/AHA guidelines for the evaluation and management of chronic heart failure in the adult: executive summary: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. Circulation 2001;104:2996–3007.
19. Luzier AB, Forrest A, Adelman M, et al. Impact of angiotensin-converting enzyme inhibitor underdosing on rehospitalization rates in congestive heart failure. Am J Cardiol 1998;82:465–9.
20. VanVeldhuisen DJ, Genth-Zothz S, Brouwer J, et al. High- versus low-dose ACE inhibition in chronic heart failure: a double-blind, placebo-controlled study of imidapril. J Am Coll Cardiol 1998;32:1811–8.
21. Jorde UP, Ennezat PV, Lisker J, et al. Maximally recommended doses of angiotensin-converting enzyme (ACE) inhibitors do not completely prevent ACE-mediated formation of angiotensin II in chronic heart failure. Circulation 2000;101:844–6.
22. Tang WHW, Vagelos RH, Yee Y-G, et al. Neurohormonal and clinical responses to high- versus low-dose enalapril therapy in chronic heart failure. J Am Coll Cardiol 2002;39:70–8.
23. London B. A second ACE of hearts (editorial). J Mol Cell Cardiol 2003;35:1009–10.
24. Mankad S, d'Amato TA, Reichek N, et al. Combined angiotensin II receptor antagonism and angiotensin-converting enzyme inhibition further attenuates postinfarction left ventricular remodeling. Circulation 2001;103:2845–50.
25. Sakata K, Yoshida H, Obayashi K, et al. Effects of losartan and its combination with quinapril on the cardiac sympathetic nervous system and neurohormonal status in essential hypertension. J Hypertens 2002;20:103–10.
26. Massie BM. Neurohormonal blockade in chronic heart failure. J Am Coll Cardiol 2002;39:79–82.
27. de Groote P, Helbecque N, Lamblin N, et al. Beta-adrenergic receptor blockade and the angiotensin-converting enzyme deletion polymorphism in patients with chronic heart failure. Eur J Heart Fail 2004;6:17–21.
28. Poole-Wilson PA, Swedberg K, Cleland JG, et al. Comparison of carvedilol and metoprolol on clinical outcomes in patients with chronic heart failure in the Carvedilol Or Metoprolol European Trial (COMET): randomized controlled trial. Lancet 2003;362:7–13.